Abnormalities in Iron Metabolism in Multiple Sclerosis

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ABSTRACT: High iron concentrations have been reported in the brains of multiple sclerosis victims. To determine if there are abnormalities in general iron metabolism indicative of iron overload in MS, measurements of transferrin saturation, serum ferritin and red cell ferritin in 31 female and 18 male patients were compared to the results in 49 age- and sex-matched healthy controls. Compared to controls, mean serum ferritin in MS was high, whereas transferrin saturation and red cell ferritin were similar. High values in one or more individual test results were observed in eleven MS patients. They were prevalent in patients who required bilateral assistance to walk or were confined to a chair, and appeared to be related to the severity of the disease. An investigation was made into the relationship of the high serum ferritin values in MS to the HLA-A_3 histocompatibility antigen, a marker of the hemochromatosis gene which is prevalent in MS. A statistically significant interaction was not found between serum ferritin and the presence of HLA-A_3.

RÉSUMÉ: Anomalies du métabolisme du fer dans la sclérose en plaques Une concentration élevée en fer a été rapportée dans le cerveau de patients atteints de sclérose en plaques (SEP). Afin de déterminer s'il existe des anomalies du métabolisme général du fer indicatives d'une surcharge en fer dans la SEP, nous avons comparé les taux de saturation de la transferrine, de ferritine sérique et de ferritine des globules rouges chez 31 femmes et 18 hommes atteints de SEP et chez 49 contrôles sains appariés pour l'âge et le sexe. Le taux moyen de la ferritine sérique dans la SEP était élevé par rapport aux contrôles, alors que les taux de saturation de la transferrine et de ferritine des globules rouges étaient semblables. Des valeurs élevées pour un test particulier ont été observées chez onze des patients avec SEP, plus particulièrement chez les patients qui avaient besoin d'appui bilatéral pour marcher ou qui étaient en fauteuil roulant, et cette élévation semblait reliée à la sévérité de la maladie. Nous avons étudié la relation entre des valeurs élevées de ferritine sérique dans la SEP et l'antigène d'histocompatibilité HLA-A_3, un marqueur du gène de l'hémochromatose ayant une fréquence élevée dans la SEP. Il n'existait pas d'interaction statistiquement significative entre la ferritine sérique et la présence du HLA-A_3.

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White matter samples from brains of multiple sclerosis (MS) victims have significantly higher concentrations of iron, calcium and zinc, as compared to controls.[1] Decreased signal intensity of T2-weighted MR images of the thalamus and putamen has been ascribed to iron deposition.[2] Stainable iron deposits have been reported surrounding plaques in the brains of MS patients by one group,[3] and not by another.[4] The first objective of this study was to determine whether there were abnormalities in general iron metabolism indicative of iron overload in patients with MS. The second objective of this study was to determine whether or not abnormalities in general iron metabolism are linked to the HLA-A_3 histocompatibility antigen, a marker for the hemochromatosis gene.[5] For example, in our hemochromatosis pedigrees, the prevalence of HLA-A_3, B_7 and B_{14} in homozygotes is 62%, 57% and 22%, respectively, compared to 25%, 22% and 8% in controls. Multiple sclerosis affects an ethnic population similar to hereditary hemochromatosis, and there is an increased prevalence of the HLA-A_3 antigen.[6]

Methods

Patients with Multiple Sclerosis

Blood samples were obtained from 49 patients (31 female and 18 male) who attended the MS clinic at University Hospital. None of the patients had clinical features of liver disease, infection, or other inflammatory conditions which are known to affect iron metabolism. The mean age was 43±11 (standard deviation) years. The HLA-A_3 antigen was present in 39 percent of the patients. Four patients with HLA-A_3 and three patients without this allele had been taking iron supplements as part of a mineral-vitamin preparation.

Controls

The results obtained in patients for transferrin saturation and serum ferritin were compared to 49 age- and sex-matched controls (Group A) selected from the Nutrition Canada Survey.[7] The results for red cell ferritin were compared to a second control

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group (B), matched for age and sex.[8] The controls ranged in age from twenty to seventy-five years. None of them had symptoms or signs of illness. Individual hemoglobin concentrations were within the normal range for men and women.

Hereditary Hemochromatosis

The patients with MS were also matched for age and sex to 49 heterozygotes who were identified by HLA typing of pedigrees with hereditary hemochromatosis.[9]

Iron Studies

Serum iron and total iron binding capacity (TIBC) were measured using a diagnostic kit manufactured by Hoffman-La Roche Ltd., Montreal. Percent transferrin saturation was determined from the ratio of serum iron/TIBC. The range of normal values was 16-55%. Serum ferritin was measured using a radioimmunoassay employing antibodies against human liver ferritin (Quant-immune Ferritin, IRMA, Biorad, Chemical Division, Richmond, California). Red cell ferritin was measured using the same commercial kit.[8]

HLA Typing

Typing for the A and B loci was performed by the complement-dependent cytotoxicity technique.[10]

Statistical Analysis

A one-tailed t-test (paired comparisons) was performed using a Hewlett Packard series 200 computer. A one-tailed test was used because we were testing whether the indices in iron metabolism were increased over controls. Frequency distribution of values for serum ferritin and red cell ferritin showed a skewed distribution, and were normalized by logarithmic transformation. Analysis of variance was performed with log-transformed data. Power analysis was done according to Armitage.[11]

Results

Iron Status of Patients with Multiple Sclerosis

The mean transferrin saturation of patients with MS was similar to that of control Group A (Table 1). Mean serum ferritin concentration in the patients, 64 ng/ml, was significantly greater than the value of 37 ng/ml in control Group A, paired t-test, $p<.01$. Red cell ferritin level in the patients, 13 ag/cell, was not significantly different from control Group B, paired t-test, $p>0.01$. Five of the individual serum ferritin values, and nine of the individual red cell ferritin results in MS, exceeded the normal range of results for these tests (Table 2). When the tests were repeated two years later, serum ferritin remained elevated in five patients, and red cell ferritin was still high in six out of nine patients. Only one of these patients, CP, was taking an over-the-counter iron supplement. The one patient, NG, with a marked fall in both serum ferritin and red cell ferritin, had developed hypothyroidism in the interim. High individual results for serum ferritin or red cell ferritin were about equally distributed between patients with HLA-A_3 and without the HLA-A_3 antigen (Table 2).

Table 1: Iron Status of Patients with Multiple Sclerosis

Designation	Number of Subjects	Transferrin Saturation (percent) Mean ± SD	Serum Ferritin (ng/ml) Geometric Mean 5th & 95th Percentile	Red Blood Cell Ferritin (ag/cell) Geometric Mean 5th and 95th Percentile
Controls A	49	24 ± 8	37 (6-206)	
B	49	—	—	10 (5-21)
Multiple Sclerosis	49	25 ±10	64[a] (6-323)	13 (4-56)

a = mean value of this group statistically significantly different from corresponding mean value of control group, $p<.01$, t-test paired comparisons.

Comparison of Iron Status of MS Patients with Heterozygous Hemochromatosis

The mean transferrin saturation, 25±10% (SD) in 49 MS patients was significantly lower than the mean value of 34±14% in 49 heterozygotes, $p<0.01$, paired t-test. Geometric mean serum ferritin in MS patients, 62 ng/ml, was not statistically different from the mean value of 81 (8-375, 5th and 95th percentile respectively) ng/ml in heterozygotes, $p>0.10$, paired t-test. Mean red blood cell ferritin in MS patients, 13 ag/cell, was statistically different from the mean value of 24 (5-71, 5th and 95th percentile respectively) ag/cell in the heterozygotes, $p<0.01$, paired t-test.

Relationship of Iron Status of MS Patients to the HLA-A_3 Antigen

Accepting the frequency of the hemochromatosis allele in the general population of 0.04-0.06, and the probability of about 0.19[12] that a chromosome carrying the A_3 allele also carries the hemochromatosis gene, it can be estimated that about 4-5 of the nineteen A_3 positive MS patients were heterozygotes, compared to 2-3 of the thirty A_3 negative MS patients.[13]

Application of a t-test for an interaction between HLA-A_3 antigen and the mean values for transferrin saturation, serum ferritin or red cell ferritin in MS patients with and without HLA-A_3, revealed no statistically significant interaction. Assuming a standard deviation of 10 units, a two-sample comparison of 49 subjects in each group has 80% power ($\alpha=.05$) of detecting a true difference in mean transferrin saturation of at least six units.[11] For red blood cell ferritin, similar calculations indicate that the two-group comparison has adequate power for detecting a true mean difference of 14 units. Thus the non-significant results for these two variables are based on sample sizes with sufficient statistical power for detecting moderately large differences.

Discussion

Serum ferritin provides a good index of the size of body iron stores in healthy subjects. Serum ferritin levels may be increased disproportionate to the size of body iron reserves in the presence of infection or liver disease. The modest increase in serum ferritin in the individual MS patients, compared to controls, was not explained by these conditions. The high serum ferritin in MS patients could represent a non-specific increase due to inflammation associated with the disease process itself.[14]

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Table 2: High Individual Results in Multiple Sclerosis Patients

Initial	HLA-A_3	Age/Sex	Hemoglobin g/L 1986	Serum Iron umol/L 1986	TIBC umol/L 1986	Transferrin Saturation (%) 1986	1988	Serum Ferritin ng/ml 1986	1988	Red Cell Ferritin ag/cell 1986	1988	Clinical Condition
HW	Yes	32F	152	30	49	61	45	323	347	90	102	deterioration
MA	Yes	33F	120	21	43	49	37	135	156	63	38	stable
KM	Yes	37F	136	11	54	21	46	47	65	32	21	deterioration
CP	Yes	58F	123	9	43	21	32	409	225	38	39	stable
JF	Yes	33M	156	17	46	37	32	129	97	36	23	stable
JB	No	45F	137	17	48	35	30	250	270	56	41	stable
NG	No	35F	142	18	58	30	12	153	9	202	7	deterioration
MS	No	51F	153	22	57	39	26	54	37	39	32	stable
RR	No	44F	133	15	47	31	—	106	—	27	—	—
NC	Yes	68M	140	16	50	33	18	596	534	22	—	stable
TW	No	38M	167	11	65	17	17	585	493	9	7	deterioration
	Normal Values		M 135-160 F 115-146	12-31	45-73	16-55		M 18-350 F 18-200		4-25		

High values were evident, however, in patients whose condition was stable, as well as in those where it was deteriorating.

Nine of the eleven MS patients with abnormalities in iron metabolism required bilateral assistance to walk or were restricted to wheelchair or chair. Normally, skeletal muscles serve as an important storage site for body iron. The total amount of iron stored here is about the same as that found in the liver and bone marrow respectively.[14] It is possible that muscle atrophy in the MS patients led to a shift of iron from muscle to the liver or reticuloendothelial system, and this contributed to the high serum ferritin values.

None of the MS patients with the HLA-A_3 marker selected for this study had increases in transferrin saturation, serum ferritin and red cell ferritin that are typical of homozygous hemochromatosis. No homozygotes have been recognized on clinical grounds in the 1,700 MS patients we have encountered. Partial biochemical expression of the hemochromatosis gene, characterized by a modest increase in mean transferrin saturation and serum ferritin, was evident in the heterozygotes selected for this study. No increase in transferrin saturation was apparent, however, in the MS patients. Although serum ferritin was high in MS patients, the increase in serum ferritin was apparent in the presence of, and in the absence of, the HLA-A_3 histocompatibility antigen (Table 2).

While there was no apparent systematic association between high serum ferritin values and the HLA-A_3 histocompatibility antigen, it is still possible that abnormalities in some individuals reflect the co-association of hemochromatosis and MS with HLA-A_3. To explore this possibility will require a method for the direct detection of the hemochromatosis gene. At present, this is not feasible.

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